CHRONIC FISH BIOASSAYS
AT
CRESTBROOK FOREST INDUSTRIES PULPMILL
IN 1987 AND 1988

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Les McDonald, RP Bio
Environmental Protection
BC Environment
Cranbrook, B.C.

BC Environment

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SUMMARY

The Crestbrook Forest Industries bleached kraft pulp mill at Skookumchuck normally discharges its secondarily treated effluent to ground via a series of rapid infiltration basins. During spring freshet, when a river colour increase requirement can be met, the discharge is direct to the Kootenay River. There was no direct discharge to the Kootenay River in 1989 and 1990. In 1986 coloured seepage from the basins began to surface below the escarpment on which the basins are situated and flow into a channel of the Kootenay River. In 1987 and 1988 the toxicity of this seepage was tested *in situ* using caged Rainbow trout fry. The 1987 study was a simple lethal toxicity test, in 1988 sub-lethal toxicity was tested by examining exposed fish for gill tissue damage and analyses were performed to test for bioaccumulation of chlorophenols.

The seepage was not lethal to any of the Rainbow trout in either a static 96 hour laboratory bioassay on the seepage itself or in a 14 day (1987) or a 40 day (1988) *in situ* test immediately downstream in the river. There were no significant histopathological effects in the gill tissue of caged fish in 1988 after 40 days. Blind coded samples from each of four sites, one upstream control and three downstream sites, and from four croppings over the 40 days showed no detectable differences or trends related to exposure to the rapid infiltration seepage.

There were no detectable quantities of any of the five chlorophenol compounds analyzed in the whole body of fish exposed for 40 days. These fish were viscerated to prevent contamination from stomach contents and removal of the liver may explain why no chlorophenols were detected since this is the organ which accumulates and metabolizes toxins. Concentrations of chlorophenols, however, were very low in the seepage and undetectable in the river just downstream. Two pairs of samples from the effluent entering the rapid infiltration basins and the seepage showed a 97% drop in total chlorophenols.

None of the chlorophenol compounds were detected in the river sediments. Several resin acids were, however, detected in the sediments, including at the upstream site. This site was above any rapid infiltration seepage but below the diffuser which had discharged effluent four months before sampling. The fish in the *in situ* bioassays were separated from the river sediment by the floor of the aluminum cages.

It is recommended that effluent to be discharged directly to the Kootenay River, which is

required by permit to be non-lethally toxic, and the seepage from the Rapid Infiltration Basins be periodically checked for sub-lethal toxicity. Hepatic Mixed Function Oxidase (MFO) induction in test fish is one of the new methods that could be used. *In situ* bioassays or native fish capture techniques are not possible for direct discharge monitoring because of high river flows. For this assessment fish could be exposed to serial dilutions of effluent in a laboratory to determine a "no effects" concentration which could be compared to estimated effluent river dilutions. The seepage could be tested during low flows using *in situ* bioassays or native fish capture. MFO monitoring of fish is being considered for inclusion in the Environmental Effects Monitoring Program (EEMP) of the Pulp & Paper Regulations under the Federal Fisheries Act. EEMP protocols should be followed for MFO monitoring at Crestbrook Forest Industries.

It is further recommended that the use of the Rapid Infiltration Basins continue until process and/or treatment system changes can be introduced that produce a less toxic effluent. Area soils appear to function well in removing toxic components of the effluent, namely chlorinated phenols and resin acids. There is some indication in the scientific literature that these compounds are dechlorinated and detoxified by anaerobic microbial activity in soils. Further study is required to determine the actual fate of these compounds beneath the Rapid Infiltration Basins.

GLOSSARY

acute toxicity - causing the death of the test organism. By convention, this is usually tested over 96 hours, some lethal tests are now being run for 7 days or longer.

BKME - bleached kraft mill effluent. The conventional bleaching process is the source of half the BOD, all the organochlorines, and most of the colour and toxicity in the effluent from typical bleached kraft pulp mills. The toxicity of bleach plant effluents is being reduced by replacing elemental chlorine with oxygen, chlorine dioxide and hydrogen peroxide. (Bonsor *et al* 1988)

chlorinated phenolics - (a.k.a. chlorophenols, chlorophenolics, chlorinated phenols) low molecular weight compounds characterized chemically by having at least one benzene ring with a hydroxyl (-OH) group attached (= hydroxy-benzene). In conjunction with chlorinated resin acids and fatty acids, these compounds account for much of the acute lethality of BKME. They are partly removed by secondary treatment, only moderately persistent in the environment, and moderately bioaccumulative in fish (readily excreted upon return to clean water). (Bonsor *et al* 1988)

chronic toxicity - also referred to as sub-lethal toxicity, does not cause the immediate death of the organism but there may be long term effects such as reduced growth or impaired reproductive capacity or the development of cancerous lesions. Testing is much more difficult and often the meaning or consequence of the measurable effect is not understood, ie. increase in liver enzyme activity.

96 hour LC_{50} - the calculated concentration of effluent which is lethal to 50% of the test fish during a 96 hour exposure. At least five different concentrations of effluent plus a control are used in the calculation.

rapid infiltration basins - large, shallow basins excavated in native soils, sized to allow one days effluent to infiltrate to ground. These were initially designed for the purpose of removing the dark brown colour from the effluent via a chemical reaction in the alkaline soils. Initial studies showed that most of the colour would be removed by the time the effluent reached the Kootenay River it would be clear. The discovery of chlorinated phenol compounds,

with their associated toxicity, has raised concerns about long-term feasibility of rapid infiltration since the fate of these compounds is largely unknown.

resin acids - naturally occurring toxic organic compounds which are part of the tree's natural defense system against insect attack. Though acutely toxic to fish, resin acids are degraded readily by secondary treatment. Some of these compounds can become chlorinated during the bleaching of pulp.

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1.0 INTRODUCTION

Crestbrook Forest Industries operates a softwood bleached kraft pulpmill at Skookumchuck on the Kootenay River, 40 km north of Cranbrook. The mill employs a modified convention bleaching sequence (C E D E D) and mill production currently averages around 580 ADT/d. Effluent flows average 45 000 to 50 000 m^3/d but treatment is somewhat unique for bleached kraft pulp mills. There is a conventional secondary level of treatment with two primary settling ponds to remove fines and a baffled aeration basin for biological treatment to reduce BOD and toxicity. At this stage of treatment the mill is presently permitted to discharge the effluent at a rate of 68,200 m^3/d to the Kootenay River providing the quality meets toxicity, BOD, and suspended solids requirements and does not raise the colour level in the river more than 15 units above background. When not discharging to the river the effluent enters a large (tertiary) polishing lagoon to remove any suspended solids (microbial in origin) and then to a series of (quaternary) rapid infiltration basins where the native fluvial soils remove the colour from the effluent before it makes its way, via groundwater flow, to the Kootenay River. The effluent colour restriction effectively means that discharge to the river can only occur during freshet from May to August or (rarely) September.

The RI basins first went into operation in November 1981 and have since functioned quite well with respect to their original purpose - to remove colour from the effluent. In 1986 seepage began to surface at the foot of the embankment below the RI Basins. This seepage continues today with a greater or lesser flow depending on which basin is being used. Presently, the seepage flow represents about 20% of the total effluent flow. As of the date of writing three basins are not being used because of the high rate of leakage and Environmental Protection has requested an examination of the geological stability of the area.

The seepage enters a side channel of the Kootenay River which rejoins the main channel just upstream of the Highway 95 bridge at Skookumchuck (see Figure 1). The discolouration of the Kootenay River is visible from the bridge except during high water and has resulted in numerous complaints and inquiries.

Because there is no requirement that the effluent discharged to the RI basins be non-toxic there was a concern that the seepage entering the Kootenay River would have an adverse impact on

aquatic life, specifically fish. In the fall of 1987, in order to investigate the potential impacts of this seepage, the Waste Management Branch (now Environmental Protection) conducted an acute *in-situ* bioassay in the vicinity of the seepage using juvenile Rainbow trout. At this time static bioassays were also performed on the seepage. Prompted by recent research findings on the sub-lethal impacts of chlorinated organic compounds the 1988 sampling program was expanded.

In the more detailed 1988 study, analysis for several chlorinated organic compounds and resin acids, commonly found in BKME, was performed on the river water, effluents, river sediments, and on the tissues of the test fish. In addition, sub-samples of the fish from each site were taken four times through the 40 day exposure period for histological examination to determine if there had been damage to the gill tissue.

2.0 METHODS

In both 1987 and 1988 the *in situ* bioassays were conducted by placing hatchery raised Rainbow trout fry (*Salmo gairdneri*) in aluminum cages anchored in the river near shore. The cages measured 140 cm x 30 cm x 30 cm and had a 30 cm x 30 cm grid of 5 mm holes on opposite sides and ends of the cage to provide river flow-through. A 30 cm x 30 cm door on the top at one end allowed observation and access for sampling. In 1988 the end opposite this door was fitted with a removable plexiglass window to make observation and sampling easier.

The cages were anchored with wooden stakes on the west bank of the river in about 30 cm of water so that the river did not wash over the door. River level fluctuation necessitated occasionally moving the cages a short distance to find the desired depth.

The location of the cage sites is shown on Figure 1. Site 1 served as a control and was located at the beginning of the west channel of the Kootenay River, above any sources of seepage. During high flow, in the spring, this site is located downstream of the effluent diffuser. In 1987 direct river discharge occured for 47 days from May 1 to May 20 and from June 6 to July 3 and in 1988 for 27 days from May 18 to June 13. Site 2 was located in the initial dilution zone of the largest point source of seepage, about 300 meters down the west channel. Site 3 was located near the mouth of the west channel, 500 meters downstream from Site 2. In 1988 a fourth site was added in the main channel of the Kootenay River, about 50 meters downstream of the Highway 3

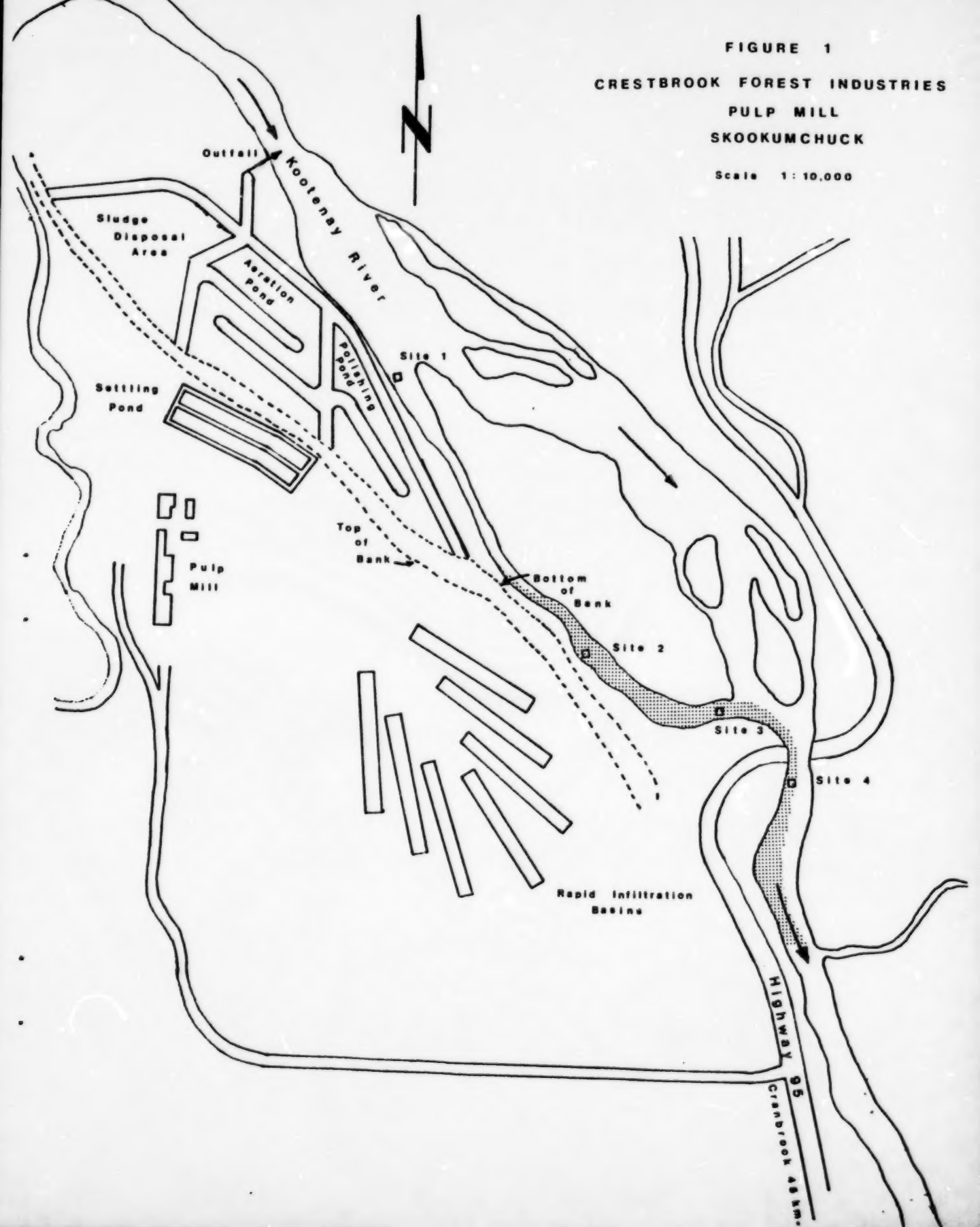
FIGURE 1

CRESTBROOK FOREST INDUSTRIES

PULP MILL

SKOOKUMCHUCK

Scale 1:10,000



bridge on the west bank.

In 1987 10 fish were placed in each of the cages at Sites 1,2,and 3 and left for 12 days from September 28 to October 9. The cages were checked daily through this period and observations made on the condition of the fish as well as measurements made for water temperature, pH, dissolved oxygen and water velocity in front of the cage. On September 28, 80 litres of the seepage was collected for a 96 hour static Rainbow trout bioassay which was conducted at the Ministry of Environment Aquatic Toxicity Laboratory in North Vancouver. On September 28 and October 6 samples of the seepage and the river at each site were taken for analysis of resin acids and BOD.

In 1988 20 fish were placed in each of the cages at Sites 1,2,3, and 4 and left for 40 days from October 6 to November 17. The average length and weight of these fish was 7.0 cm and 3.7 g giving an average condition factor of 1.07 ($K = Wt \times 100 / length^3$). Due to the lack of mortalities in 1987, the cages were checked less frequently, nine times throughout the 40 day period. At four intervals over the 40 days 4 fish were cropped from each cage and preserved for histological examination to determine if any tissue damage had occurred. Croppings were done on day 12, day 21, day 32 and day 40. These fish were removed from the cages with a dip net and placed in an anaesthetizing solution of MS 222 at 75 mg/L. After the fish had become immobilized they were transferred to a killing solution of MS 222 at 150 mg/L. After about 10 minutes in this solution, when all movement had ceased, the gill opercula and one abdominal wall were carefully dissected away. The fish were then placed in jars of 10% buffered, neutralized formalin and labelled with a meaningless number code so that the examining laboratory staff had no knowledge of the experimental conditions. Histological examination was performed by the B.C. Ministry of Environment, Fish Health Laboratory at the Pacific Biological Station in Nanaimo.

On day 5, day 25 and day 40 samples of the seepage and river water at each cage site were analyzed for seven resin acids (see Table 4) and the five selected organochlorine compounds listed below:

2,4,6 - Trichlorophenol
2,3,4,6 - Tetrachlorophenol
Pentachlorophenol
4,5,6 - Trichloroguaiacol
Tetrachloroguaiacol

Note on analysis :

Methylation prior to analysis of the chloroguaiacols meant that chlorocatechols and chloroveratrols were also analyzed (D.Hope, personal communication). For example, reported values for 4,5,6 -trichloroguaiacol are actually a total of 4,5,6 -trichloroguaiacol, 4,5,6 -trichlorocatechol, and 4,5,6 - trichloroveratrol. This should be kept in mind when guaiacols are referred to throughout this report.

On day 25 and day 40 these analyses were also performed on the effluent feed flowing into the RI basins. On day 14 three replicate samples of river bottom sediment were taken from around each of the cages and analyzed for the same parameters. Finally, on day 40 three replicate fish were taken from each cage plus three control fish from the Hatchery and frozen for whole body analysis for the organochlorine compounds. Resin acid analysis could not be performed by the Laboratory due to interference by naturally occurring fatty acids (S. Brynjolfson, pers. comm.). The fish were viscerated prior to freezing so that gut contents would not contaminate the tissue analyses.

3.0 RESULTS

To insure that general conditions in the cages were not affecting the condition of the fish and were similar between sites, field measurements for temperature, dissolved oxygen, pH, and water velocity were taken during inspection visits. The results of temperature and dissolved oxygen measurements taken inside the cages are listed in Tables 1 and 2. In 1987 temperatures ranged between 6° and 10° and were very similar between stations. In 1988, by mid-November temperatures had ranged from 9° down to 1.5°. Dissolved oxygen in both years ranged from 7.8 mg/L to 10.4 mg/L and never reached levels that could be considered stressful to fish. In 1987 dissolved oxygen was measured inside and outside the cage. Levels just in front of the cages were consistently 0.4 to 1.0 mg/L higher than in the cages at all sites.

The pH at all sites in 1987 ranged between 7.8 and 8.3 while in 1988 it ranged from 8.1 to 8.6. This discrepancy was probably due to instrument error. In both years the west channel stations (2 and 3) were always 0.2 to 0.4 units lower than the Kootenay River stations (1 and 4).

An attempt was made, by adjusting the onshore/offshore location of the cages, to keep them in a flow of about 12 cm/s (0.4 ft/s). This was only a problem at cage 2 in the west channel where flows occasionally dropped to 3 cm/s. Other field measurements, however, suggest that

Table 1. Water Temperature (° C) in Cages

Year	Day	Upstream	Below Seepage	West Channel	Kootenay R
87	1	10	10	10	ND
87	2	10	10	10	ND
87	3	9	10	10	ND
87	4	10	9	10	ND
87	5	9	10	9	ND
87	6	9	9	9	ND
87	7	8	9	8.5	ND
87	8	8	9	8.5	ND
87	9	8	8	8.5	ND
87	10	7.5	8	8	ND
87	11	7.5	7.5	8	ND
87	12	6	6.5	7.5	ND
88	1	9	9	9	10
88	9	8.5	9	9.2	8.2
88	13	5.5	5.8	6	6
88	22	4	4	4.5	4.5
88	33	4	4.2	5.2	5
88	43	1.5	2	4	2.5

Table 2. Dissolved Oxygen in Cages (mg/L)

Year	Day	Upstream	Below Seepage	West Channel	Kootenay R
87	1	9.7	9.5	9.3	ND
87	2	8.7	8.9	8.6	ND
87	3	9.1	8.7	9.3	ND
87	4	8.8	8.7	8.8	ND
87	5	8.3	7.5	7.8	ND
87	6	8.3	8.0	8.1	ND
87	7	8.4	7.9	8.3	ND
87	8	8.6	8.1	8.1	ND
87	9	9.3	8.4	8.5	ND
87	10	8.8	8.5	8.4	ND
87	11	9.7	8.3	8.4	ND
87	12	9.6	8.5	8.8	ND
	mean	8.9	8.4	8.5	
88	1	10.2	9.8	9.3	10.0
88	6	9.7	9.9	9.6	10.4
88	9	9.4	8.6	9.0	8.6
88	13	9.7	8.7	9.2	9.0
88	22	9.7	7.8	9.1	9.5
88	33	10.1	9.4	9.0	8.5
88	43	10.6	10.2	9.5	9.8
	mean	9.9	9.2	9.2	9.4

ND = Not Done

this was not a problem for the fish.

3.1 River and Effluents

Analyses for the five chlorinated organic compounds listed in Section 2.0 can be found in Table 3. Most notable is the fact that none of the compounds were detected in the river, whether in the main channel or in the west channel just below the seepage, where discolouration was obvious. Tetrachloroguaiacol (TTCG) was found in all three seepage samples with concentrations ranging from 0.001 mg/L to 0.007 mg/L. Only two samples of the mill effluent being discharged to the RI basins were taken in October and November 1988. The concentrations of 2,4,6-Trichlorophenol (TCP), 2,3,4,6-Tetrachlorophenol (TTCP), and TTCG in these two samples varied by as much as an order of magnitude. The order-of-magnitude increase in these compounds in the effluent over 17 days did not seem to be translated directly into similar increases in the seepage. On October 18, 1988 the TTCG in the effluent was 0.044 mg/L and 0.001 mg/L in the seepage. On November 17, 1988 the concentration in the effluent had risen to 0.15 mg/L while the level in the seepage was only 0.002 mg/L. Similar results were found for TCP. Changes in the concentration of these compounds in the RI seepage compared to the RI feed is further complicated by the fact that different basins have different lengths of paths through the soils. Regardless of the variation in the proportions of each compound, the totals of all compounds analyzed showed a 97% drop from the RI feed to the seepage in both pairs of samples.

The resin acid results are listed in Table 4. While all seven resin acids were found in significant concentrations in the RI feed, only dehydroabietic acid (DHA) was found in the seepage. The total resin acid (TRA) concentration in the effluent ranged between 9.83 mg/L and 13.30 mg/L (n=2, 1988) while in the seepage levels ranged from 0.019 mg/L to 0.240 mg/L (n=5, 1987 and 1988). Paired values in 1988 showed 98 to 100 % reduction in TRA from the RI feed to the seepage.

3.2 River Sediments

Three replicate sediment samples were taken on October 20, 1988 at each of the cage sites. Of a total of twelve samples, none of the chlorophenol compounds were detected at a MDC of

Table 3. Chlorinated Organics in Effluents and Kootenay River

Site	Date	Tri CP	Tetra CP	Penta CP	Tri CG	Tetra CG
		mg/L	mg/L	mg/L	mg/L	mg/L
Inlet of West channel (Cage 1)	10/11/88	ND	ND	ND	ND	ND
Inlet of West channel (Cage 1)	10/31/88	ND	ND	ND	ND	ND
Inlet of West channel (Cage 1)	11/17/88	ND	ND	ND	ND	ND
RI Feed	10/11/88	NS	NS	NS	NS	NS
RI Feed	10/31/88	0.019	0.0220	0.0023	0.34	0.044
RI Feed	11/17/88	0.270	0.0066	0.0029	0.35	0.150
RI Seepage	10/11/88	ND	ND	ND	ND	0.007
RI Seepage	10/31/88	ND	ND	ND	0.012	0.001
RI Seepage	11/17/88	0.0003	0.0001	0.0002	0.019	0.002
Just Below Seepage (Cage 2)	10/11/88	ND	ND	ND	ND	ND
Just Below Seepage (Cage 2)	10/31/88	ND	ND	ND	ND	ND
Just Below Seepage (Cage 2)	11/17/88	NS	NS	NS	NS	NS
West channel mouth (Cage 3)	10/11/88	ND	ND	ND	ND	ND
West channel mouth (Cage 3)	10/31/88	ND	ND	ND	ND	ND
West channel mouth (Cage 3)	11/17/88	ND	ND	ND	ND	ND
Skookumchuck bridge (Cage 4)	10/11/88	ND	ND	ND	ND	ND
Skookumchuck bridge (Cage 4)	10/31/88	ND	ND	ND	ND	ND
Skookumchuck bridge (Cage 4)	11/17/88	ND	ND	ND	ND	ND

ND = Not Detected (MDC for phenols = 0.0001 mg/L, guaiacols = 0.001 mg/L), NS = Not Sampled

Table 4. Resin Acids in Effluents and the Kootenay River

Site	Date	Abietic	Dehydroabietic	Isopimaric	Levopimaric	Neoabietic	Pimaric	Sandaracopimaric	Total RA
		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Inlet of West channel (Cage 1)	9/28/87	ND	ND	ND	ND	ND	ND	ND	ND
Inlet of West channel (Cage 1)	10/6/87	ND	ND	ND	ND	ND	ND	ND	ND
Inlet of West channel (Cage 1)	10/11/88	ND	ND	ND	ND	ND	ND	ND	ND
Inlet of West channel (Cage 1)	10/31/88	ND	ND	ND	ND	ND	ND	ND	ND
Inlet of West channel (Cage 1)	11/17/88	ND	ND	ND	ND	ND	ND	ND	ND
RI Feed	10/11/88	NS	NS	NS	NS	NS	NS	NS	NS
RI Feed	10/31/88	0.97	5.2	2.1	0.046	0.10	1.2	0.22	9.83
RI Feed	11/17/88	2.1	6.0	2.8	0.46	0.32	1.3	0.31	13.30
RI Seepage	9/28/87	ND	0.048	ND	ND	ND	ND	ND	0.048
RI Seepage	10/6/87	ND	0.019	ND	ND	ND	ND	ND	0.019
RI Seepage	10/11/88	<0.004	0.026	<0.004	<0.004	<0.004	<0.004	<0.004	0.026
RI Seepage	10/31/88	ND	ND	ND	ND	ND	ND	ND	ND
RI Seepage	11/17/88	<0.008	0.24	<0.008	<0.008	<0.008	<0.008	<0.008	0.240
Just Below Seepage (Cage 2)	9/28/87	ND	ND	ND	ND	ND	ND	ND	ND
Just Below Seepage (Cage 2)	10/6/87	ND	0.003	ND	ND	ND	ND	ND	0.003
Just Below Seepage (Cage 2)	10/11/88	NS	NS	NS	NS	NS	NS	NS	NS
Just Below Seepage (Cage 2)	10/31/88	ND	ND	ND	ND	ND	ND	ND	ND
Just Below Seepage (Cage 2)	11/17/88	NS	NS	NS	NS	NS	NS	NS	NS
West channel mouth (Cage 3)	9/28/87	ND	ND	ND	ND	ND	ND	ND	ND
West channel mouth (Cage 3)	10/6/87	ND	0.002	ND	ND	ND	ND	ND	0.002
West channel mouth (Cage 3)	10/11/88	ND	ND	ND	ND	ND	ND	ND	ND
West channel mouth (Cage 3)	10/31/88	ND	ND	ND	ND	ND	ND	ND	ND
West channel mouth (Cage 3)	11/17/88	ND	ND	ND	ND	ND	ND	ND	ND
Skookumchuck bridge (Cage 4)	10/11/88	ND	ND	ND	ND	ND	ND	ND	ND
Skookumchuck bridge (Cage 4)	10/31/88	ND	ND	ND	ND	ND	ND	ND	ND
Skookumchuck bridge (Cage 4)	11/17/88	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not Detected (MDC = 0.001 mg/L), NS = Not Sampled

0.005 µg/g.

The same samples were also analyzed for seven resin acids and the results are listed in Table 5. The most abundant resin acids found were DHA and isopimaric acid. The variation between replicates at a given site was often greater than the variation between sites indicating considerable heterogeneity in the distribution of resin acids in the sediments. The highest values in any replicate were found at the cage 3 site where the DHA and isopimaric concentrations were 7.2 and 7.6 µg/g respectively and the TRA was 17.09 µg/g, an order of magnitude higher than any other replicate. No attempt was made to sample a certain particle size, except to exclude larger rocks. If the resin acids are associated with fine sediment, the proportions of this size fraction in each replicate may greatly effect the quantities extracted and detected.

There appears to be a broad distribution of resin acids in the sediments throughout this area of the river with considerable quantities found even at the control site. This control site was above active seepage but below the direct discharge outfall. Direct discharge to the Kootenay River in 1988 had ceased on June 13 which indicates that these compounds persist in the river sediments for at least four months.

3.3 Fish

Analysis of the 3 replicate fish (viscerated whole body) per cage after 40 days of exposure showed no detectable levels of the chlorophenols tested. All compounds were analyzed to a MDC of 1.0 µg/g except tetrachloroguaiacol which had a MDC of 2.0 µg/g.

The acute lethality of the seepage was tested in 1987 using Rainbow Trout 96 hr static bioassays. Samples of the main seepage flow, where it entered the Kootenay River, taken on September 28 and October 6, produced no mortalities.

Monitoring for chronic or sub-lethal effects in this study involved histopathological examination of the gill tissue of exposed fish. The use of blind coding of all samples insured that any spatial (between station) or temporal (cropping after 12, 21, 32, and 40 days of exposure) trends would not be biased by anticipated effects. After examination of 68 fish in 17 samples (4 fish in 4 croppings from each cage plus 4 fish in one sample of hatchery stock), the Fish Health

Table 5. Resin Acids in River Sediments

Site	Replicate	Abietic	Dehydroabietic	Isopimaric	Levopimaric	Neoabietic	Pimaric	Sandaracopimaric	Total RA
		ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g	ug/g
Inlet of West channel (Cage 1)	1	ND	0.48	0.29	ND	ND	ND	ND	0.77
Inlet of West channel (Cage 1)	2	ND	1.0	0.69	ND	ND	0.12	ND	1.81
Inlet of West channel (Cage 1)	3	ND	0.23	ND	ND	ND	ND	ND	0.23
Just Below Seepage (Cage 2)	1	ND	0.66	0.13	ND	ND	ND	0.10	0.89
Just Below Seepage (Cage 2)	2	ND	ND	ND	ND	ND	ND	ND	ND
Just Below Seepage (Cage 2)	3	ND	1.0	0.49	ND	ND	0.05	0.10	1.54
West channel mouth (Cage 3)	1	0.43	7.2	7.6	ND	0.54	0.40	0.92	17.09
West channel mouth (Cage 3)	2	ND	0.04	ND	ND	ND	ND	ND	0.04
West channel mouth (Cage 3)	3	ND	0.55	0.10	ND	ND	ND	ND	0.65
Skookumchuck bridge (Cage 4)	1	ND	0.06	ND	ND	ND	ND	ND	0.06
Skookumchuck bridge (Cage 4)	2	ND	0.21	ND	ND	ND	ND	ND	0.21
Skookumchuck bridge (Cage 4)	3	ND	0.20	0.07	ND	ND	ND	ND	0.27

All samples taken October 20, 1988

Biologist concluded that, although some abnormalities were found, there was no correlation between these histological findings and the treatment (exposure to the seepage) and therefore the treatment had no detectable effect on the gills of the fish (S. Goldes personal communication).

4.0 DISCUSSION

The two most important findings of this study were:

1. the one to four order of magnitude reduction in chlorinated phenols and resin acids from the RI feed to the still highly coloured seepage entering the Kootenay River.

2. the lack of lethal or sub-lethal toxicity of this seepage to caged fish.

The low concentration of chlorinated phenols and resin acids in the seepage would seem to explain the lack of toxicity to the fish in these *in situ* bioassays. Bonsor et al 1988 have calculated the toxicity units (concentration in effluent/LC50) of most of the known chlorinated phenols and resin acids for biotreated bleached kraft mill effluent and found that they totalled 1.1. A total of greater than 1.0 is considered lethally toxic which means that secondary or biotreated bleached kraft mill effluent is marginally toxic. It is not surprising then that no lethal toxic effects were found in the seepage from the RI at Crestbrook (96 hour static Rainbow trout bioassay) since the concentration of many of these compounds was reduced by several orders of magnitude during the passage through the soil. Further dilution of the seepage in the river at Cage 2, which was within 10 meters of the largest seepage channel, and where the chlorinated phenols and resin acids could not be detected, did not produce any histopathological effects in gill tissue over a 40 day exposure. Couillard et al (1988) did find gill damage in caged fish after 40 and 60 day exposures to dilute untreated bleached kraft effluent with a LC_{50} of about 12%. The sub-lethal tests were performed using dilutions equivalent to 0.10 and 0.25 x the LC_{50}. Our *in situ* tests were dilutions of a well treated effluent, the RI seepage, which itself was found to be non-lethally toxic.

The attenuation of significant quantities of chlorinated phenols in the short path through the soils that the seepage travels may be the result of microbial degradation which has been demonstrated and observed many times in recent scientific research (Baker et al 1980, Boyle 1989). Many factors influence the degradation process in soils. Adsorption and persistence are

favoured by low pH, high pK_a (dissociation of acids found in soil), low DO and low organic content (Garrett and Simpson 1988). The actual structure of the pollutant also an important factor influencing biodegradation in soil and some compounds may only partly degrade leaving toxic metabolites of unknown fate (Suflita 1989). To understand the degradation process at this site, the critical conditions required, the end products and fate of any persistent compounds, will require a detailed and comprehensive study.

Sampling in 1989 showed 17 and 18 mg/L AOX in the RI feed (n=2) and 14 - 17 mg/L AOX in the seepage (n=3). The dramatic reduction in chlorophenol compounds between these two points in 1988 was apparently not accompanied by a similar reduction in AOX but the results of the 1987 and 1988 bioassays indicate that the compounds making up the AOX in the seepage were not particularly toxic.

4.1 Cautions for Interpretation of Results

Although concentrations of chorophenols and resin acids were very low in the water that the fish were exposed to, quantities of resin acids were found in the sediments. Although some sediment washed into the cages, the fish were for the most part separated from the bottom sediments by the solid aluminum walls. This effectively protected the fish from toxins in one compartment of the environment being assessed.

The lack of histological response in the gill tissue of the test fish over 40 days of exposure provides one indication that the seepage entering the Kootenay River in 1989 was not having a chronic toxic effect on fish. It must be emphasized that this is one of many indicators of chronic toxicity. Contemporary research has found many other sub-lethal effects in fish caused by BKME. Chronic stress has important implications at both the individual and community levels. An organism under chronic stress from a toxicant will have to expend energy to metabolize the compound and as a consequence suffer impaired growth and reproduction (Giesy and Graney 1989). Sprague and Colodey (1989) have summarized an extensive literature review of various chronic effects into the following categories:

Biochemical metabolites
Histopathology
Blood
Body composition

Respiration and circulation	Growth
Food conversion	Swimming stamina
Early development and reproduction	Behaviour

It is possible that other physiological effects did occur in the test fish, despite the lack of histopathological response in the gill tissue. In addition, while 40 days is a reasonably long exposure period for a bioassay, native fish may suffer sub-lethal effects from constant exposure. Fish do not exhibit a strong avoidance to BKME unless it is acutely lethal (Bonsor et al 1988, Sprague and Colodey 1989). During the fall of 1988 many kokanee were seen spawning on gravel bars throughout the west channel of the Kootenay River, which was highly coloured by the seepage at the time. The early life stages of the offspring of these fish would be exposed to the effluent for considerably longer than 40 days.

Another interesting result of this study was the apparent lack of bioaccumulation of the chlorophenol compounds in the exposed fish. Tana (1988) reported elevated levels of 2,4-dichlorophenol and 2,4,6-trichlorophenol in fish tissue after controlled exposure experiments. The highest levels were always found in the liver. In our whole fish analyses the viscera, including liver, were removed to prevent contamination from stomach contents. Tana (1989) reported muscle levels of 2,4-dichlorophenol plus 2,4,6-trichlorophenol of 0.2 µg/g after 35 days exposure and 0.8 µg/g after 80 days at 5 µg/L exposure concentration. At Cage 2 (the highest exposure) there was < 0.1 µg/L 2,4,6-trichorophenol in the river water and nothing was detected in the muscle at 1.0 µg/g MDC after 40 days. Although no chlorophenols were detected in the river sediments, this may be a possible pathway to native fish. As previously discussed, the use of *in situ* cages isolates the test fish from the river sediments unless the cages are specially designed to overcome this problem (Grizzle et al 1988). The absence of chlorophenol bioaccumulation in this experiment was therefore influenced by a combination of factors: high tissue MDC's, the removal of the livers where most of the accumulation occurs, insufficient length of exposure, and possibly lack of exposure to river sediments. Some accumulation of these compounds may be occurring in the native fish that inhabit the west channel. Tana (1988) reported similar tissue accumulation results for the dehydroabietic acid, which was found at a concentration of 3 µg/L in the river at Cage 2. Research has shown that fish can metabolize these compounds and resist chronic low levels of exposure but the expenditure of energy for detoxification may cause other systems to suffer , e.g. decreased reproductive capability and decreased growth (Bonsor et al 1988, Tana 1988).

One other factor which may have influenced the accumulation of chlorinated phenols in the test fish was their nutritional status. In both 1987 and 1988 the test fish were not fed, their only source of food being drifting invertebrates and detritus that found its way into the cages. After 40 days in 1988 the exposed fish were noticeably emaciated by comparison to hatchery fish that were fed through the same period. Nutritional status can greatly effect the accumulation, metabolism and depuration of toxins. Feeding can increase depuration and provide energy for mixed function oxidase enzyme activity which may lower tissue levels of toxins, while starvation will lower lipid reserves where some toxicants are stored (Lanno et al 1989). There is an ongoing discussion in the scientific literature about standardizing diet formulations and feeding rates and the advantages and disadvantages of monitoring test organism nutritional status (lipid content etc.). In an upstream/downstream study such as this one, the effects were presumably the same at all stations.

4.2 Implications for the Future

While not definitive, the findings of this study provide a level of assurance that the seepage reaching the Kootenay River in 1987 and 1988 was not having serious toxic effects on fish. By 1990 the seepage flow had increased considerably, not only in quantity, from 10 % of the total effluent flow in 1988 to 20% in 1990, but in the width of the plume. Seepage now passes under the RI polishing pond and enters farther up river near the mouth of the west channel. The potential impact of this greater quantity of effluent in the river has been offset by a reduction in AOX in the effluent brought about by a 40% substitution of ClO_2 for chlorine in the bleach plant first instituted in 1990. Because of the changes that have occurred in effluent quality and the quantity of seepage entering the river the 1988 findings may not be completely applicable in 1990 and beyond.

Comparisons of quantities of specific chlorophenols in the RI feed and seepage between our 1988 sampling and McKay (1990) are complicated by different analytical approaches and the large variance in the concentrations of these compounds in the effluent over time. Of all the chlorinated organic compounds in BKME, the low molecular weight chlorophenolics have shown greater toxicity to aquatic life than the high molecular weight chlorolignins, though these compounds are not particulary persistent in the environment, having half-lives of days or weeks (Bonsor et al 1988). Neither study analyzed the RI feed or seepage for the complete list of the known chlorophenolics and derivatives. A more complete analysis should include all isomers of the following:

phenols	anisoles
guaiacols	benzenes
catechols	benzoquinones
veratrols	hydroquinones
syringols	resorcinols
thiophenes	chlorinated resin and fatty acids

(Annergren et al 1987, Carlberg et al 1988, Garrett and Shrimpton 1988, Knuutinen et al 1990, Tana 1988)

Despite the differences in methodologies, this study and McKay (1990) found a reduction in chlorinated phenols from the RI feed to the seepage entering the Kootenay River. The attenuation of these toxins in the soil is important in providing maximum protection to aquatic life in the Kootenay River until new technologies and in-plant changes can be introduced that will result in a better quality effluent. Year around use of the RI, which began in 1988, has resulted in a doubling of the short circuit seepage entering the west channel. Two basins nearest the escarpment are no longer used and there is now concern that a failure or slide could occur. For the present, the mill will need to continue to use a combination of RI ground disposal during low flow and river discharge during freshet in a manner authorized in permit PE 240. Seasonal resting of the RI by discharging directly to the river appears to be necessary to insure that area soils do not become hydraulically saturated.

When discharging to the river, the mill is required to have a non-acutely toxic effluent, as defined by no more than 50% mortality of Rainbow trout in 100% effluent over 96 hours (Waste Management Permit PE 240). Even if this discharge is not acutely toxic, a major question remains: What are the chronic or sub-lethal effects on aquatic life (fish)? There is at present no universally accepted indicator of chronic stress in fish. One of the most promising methods to date is the induction of mixed function oxidase (MFO) liver enzymes. Fish (and other aquatic organisms) use these enzymes to metabolize aromatic hydrocarbons with two or more benzene rings. A significant increase in the activity of 7-ethoxyresorufin deethylase (EROD) indicates that the organism is attempting to cope with an exposure to such a toxin. In addition to the cost to the organism in expenditure of energy, the enzyme/substrate reaction involves the use of a free oxygen radical which can itself cause tissue damage (B.E. Imber, personal communication).

Recent studies have found increased levels of MFO activity in fish exposed to BKME of from 5 to 18 fold over control fish (Munkittrick et al 1990, Hodson et al, 1990). In addition to MFO induction researchers are finding a variety of effects on growth and sexual maturation. Munkittrick et al (1990) found suckers in the vicinity of a mill in Jackfish Bay on Lake Superior exhibited increased MFO activity but showed no effects on fecundity, fertilization rate, hatch, survival or development rate of larvae. Whitefish in this area, however, seemed to be exhibiting retarded sexual maturation. Hodson et al (1990) found a 5 to 10 fold increase in MFO activity in white sucker below a bleached kraft pulp mill on the St. Maurice River in Quebec. The enzyme activity eventually returned to normal with increased dilution at downstream sites. Increased liver size, hematocrit, serum glucose and serum protein followed a similar pattern. Gonad size and hormone levels (indicators of reproductive system effects) did not show any effects until 35 to 100 km downstream. The reasons for this 'downstream effect' are not yet known.

MFO testing on mill effluents using naive fish exposed to serial dilutions in upstream water could be used to develop a "no effects" threshold concentration. Mapping this dilution isopleth in the receiving environment would then identify a zone within which sub-lethal toxic effects could occur, were fish to remain in this area for a sufficient period of time.

5.0 RECOMMENDATIONS

1. An evaluation should be made of the sub-lethal toxicity of the bio-treated effluent that is discharged to the Kootenay River (ie. effluent that meets LC_{50} requirements). Assuming a valid, practical monitoring technique can be found, this evaluation should be done on a regular basis (annually). Laboratory MFO exposure of naive fish to various dilutions of the effluent is one method that could be used to determine a "no effects" concentration (dilution). Capture and examination of non-migratory sentinel species (suckers) could also be done upstream and downstream of the mill near the end of or soon after seasonal discharge. Because discharge only occurs during high flows there may be logistical problems working in the river on native fish. Measurements of the effects on growth and reproduction should also be taken, such as gonad and hepatic somatic indices (gonad and liver weight divided by body weight) as well as pathological effects such as the presence of lesions and fin ray asymmetry.

2. In a similar fashion, the sub-lethal toxicity of the RI seepage as it enters the west channel of the Kootenay River should be evaluated and regularly monitored. With the present treatment system, aeration in the bio-basins is reduced when discharging to the Rapid Infiltration Basins. The RI feed is thus quite lethal to fish and there is thus the potential for the RI seepage to be toxic to some degree despite the findings of this study.

3. Notwithstanding the outcome of the two above evaluations, the use of the Rapid Infiltration Basins should continue until mill process and waste treatment changes are made that produce a much better quality effluent. This will provide the aquatic life in the Kootenay River with the maximum protection particulary during lower flows.

6.0 REFERENCES

Annergren, G., K.P. Kringstad, and K.-J. Lehtinen. 1987. Environmental Risks Involved in Discharging Spent Bleach Liquors into Receiving Waters. P. 40-49 in Proc. EUCEPA Symposium, Helsinki, Finland, May 19-22, 1986.

Baker, M.D., C.I. Mayfield, and W.E. Inness. 1980. Degradation of Chlorophenols in Soil, Sediment and Water at Low Temperature. Water Res. 14: 1765-1771.

Boyle, M. 1989. The Environmental Microbiology of Chlorinated Aromatic Decomposition. J. of Environ. Qual. 18: 395-402.

Bonsor, N., N. McCubbin, J.B. Sprague. 1988. Kraft Mill Effluents in Ontario. Pulp and Paper Sector of MISA, Ontario Min. of Environment, Toronto, Ont.

Brynjolfson, S. 1987. B.C. Environmental Laboratory. Personal Communication.

Carlberg, G.E., S. Johnsen, L.H. Landmark, B.-E. Bengtsson, B. Bergstrom, J. Skramstad, and H. Storflor. 1988. Investigations of Chlorinated Thiophenes: A Group of Bioaccumulable Compounds Identified in the Effluents From Kraft Bleaching. Wat. Sci. Tech. 20: 37-48.

Couillard, C.M., R.A. Berman and J.C. Panisset. 1988. Histopathology of Rainbow Trout Exposed to a Bleached Kraft Pulp Mill Effluent. Arch. Environ. Contam. Toxicol. 17: 319-323.

Garret, C.L. and J.A. Shrimpton. 1988. Chemicals in the Environment: V. Chlorophenols. Environmental Protection, Env. Can. 48 p.

Giesy, J.P. and R.L. Graney. 1989. Recent Developments in and Intercomparisons of Acute and Chronic Bioassays and Bioindicators. Hydrobiologia 188/189: 21-60.

Goldes, S. 1989. B.C. Fish Health Lab, Pacific Biological Station. Personal Communication.

Grizzle, J.M., S.A. Horowitz, and D.R. Strength. 1988. Caged Fish as Monitors of Pollution: Effects of Chlorinated Effluent fro a Wastewater Treatment Plant. Wat. Res. Bull. 24: 951-959.

Hodson, P.V., M. McWhirter, K. Ralph, B. Gray, M. Gagnon, D. Thirvierge and J. Carey. 1990. Effects of Bleached Kraft Mill Effluent on Fish in the St. Maurice River, Quebec. Proc. 17th Annual Aquatic Toxicity Workshop, Vancouver, B.C., Nov. 5-7, 1990.

Hope, D. 1989. Zenon Environmental Laboratories. Personal Communication.

Imber, B.E. 1991. CBR International. Personal Communication.

Knuutinen, J.H. Palm and H. Hakala. 1990. Polychlorinated Phenol and their Metabolites in Soil and Earthworms of Sawmill Environment. Chemosphere 20: 609-623.

Lanno, R.P., B.E. Hickie and D.G. Dixon. 1989. Feeding and Nutritional Considerations in Aquatic Toxicology. Hydrobiologia 188/189: 525-531.

McKay, J.L. 1990. The Fate of Chlorinated Organics Through the Rapid Infiltration System. Crestbrook Forest Industries Ltd. Cranbrook, B.C.

Munkittrick, K.R., C. Portt, G.J. Van der Kraak, M.E. McMaster, D.G. Dixon, I.R. Smith and D. Rokosh. 1990. Characteristics of Fish Populations in a Lake Superior Bay Receiving Bleached Kraft Mill Effluent. Proc. 17th Annual Aquatic Toxicity Workshop, Vancouver, B.C., Nov. 5-7, 1990.

Sprague, J.B. and A.G. Colodey. 1989. Toxicity to Aquatic Organisms of Organochlorine Substances in Kraft Mill Effluents. Conservation and Protection, Env. Canada. 53 p.

Suflita, J.M. 1989. Microbial Ecology and Pollutant Biodegradation in Subsurface Ecosystems. In: Transport and Fate of Contaminants in the Subsurface. EPA/625/4-89/019. pp.67-84.

Tana, J.J. 1988. Sublethal Effects of Chlorinated Phenols and Resin Acids on Rainbow Trout, (*Salmo gairdneri*). Wat. Sci. Tech. 20: 77-85.